ISSN 0096-3925, Moscow University Biological Sciences Bulletin, 2018, Vol. 73, No. 1, pp. 7–12. © Allerton Press, Inc., 2018.
Original Russian Text © P.V. Zelenikhin, A.S. Gorbunova, K. Beuerlein, A.V. Makeeva, O.N. Ilinskaya, 2018, published in Vestnik Moskovskogo Universiteta, Seriya 16: Biologiya, 2018, Vol. 73, No. 1, pp. 10–15.

CELL BIOLOGY

Binase-Induced Changes of Tumor Cell Membranes

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Received October 9, 2017; in final form, December 12, 2017

Abstract—Exogenous ribonucleases of *Bacilli* can selectively induce apoptosis of malignant cells. The ability of *Bacillus pumilus* ribonuclease, binase, to induce processes leading to a dynamic disruption of the integrity of A549 human pulmonary adenocarcinoma cell membranes was analyzed. The influence of different enzyme concentrations on the state of the cytoplasmic membrane of cells and mitochondrial membranes was characterized. Using the methods of flow cytofluorometry and fluorescence microscopy, it has been established that binase leads to disruption in normal functioning of both types of membranes, with mitochondrial membranes affected first. The study made it possible to identify and visualize the effects of binase on the membrane structures of target cells and to confirm that bacterial RNase induces apoptosis of target cells mainly through the "internal" (mitochondrial) pathway.

Keywords: RNase, *Bacillus pumilus*, binase, antitumor activity, membranes disruption, pulmonary adenocarcinoma A549.

DOI: 10.3103/S0096392518010091

INTRODUCTION

Currently, close attention is paid to the biological effects of ribonuclease enzymes (RNases) that may not depend on their direct catalytic functions, such as participation in regulatory cell systems, control of the growth of blood vessels, toxicity to tumor cells, and protection from viral and microbial pathogens. Of particular interest are the RNases of organisms that are phylogenetically distant from humans and, as a result, are not affected by the RNase inhibitor in mammalian cells: RNases of cold-blooded vertebrates [1], fungi [2], and bacteria [3]. Due to the selective cytotoxic action against malignant cells, *Bacillus pumilus* RNase (binase) can be considered as a potential antitumor agent. A selective effect of binases on the human pulmonary carcinoma cell line A549 [4], fibroblasts expressing the *v-Ras* oncogen [5], myeloid leukemia cells [6], and breast cancer cells, including triple-negative ones [7], have been established. RNases at high concentrations have cytotoxic [8, 9] and genotoxic effects [10], and they also inhibit the activity of Ca-dependent potassium channels (K_{Ca} channels), leading to apoptosis [11]. Inhibition of K_{Ca} channel activity is accompanied by the appearance of morphological markers of apoptosis (cytoplasmic vacuolation, chromatin condensation and fragmentation, and cell volume reduction). However, the existing data set lacks information on the dynamics of changes in the functional state of tumor cells membranes under the action of binase.

The purpose of this work was to identify gradual changes in the state of the cytoplasmic membrane (CPM) and mitochondrial membranes of the pulmonary adenocarcinoma cell line A549 when treated with binase.

MATERIALS AND METHODS

Enzyme. This work was carried out using binase, guanyl-specific RNase of *Bacillus pumilus* (molecular weight 12.3 kDa, pI = 9.5, 109 amino acid residues) in high concentrations (100–500 μg/mL), for which apoptosis-inducing action on several cancer cell lines was previously established [4, 6, 7]. Binase was isolated as a homogeneous protein from the culture fluid of the producer according to the procedure described by Dudkina et al. [12]. The catalytic activity of binase has been characterized earlier with respect to synthetic substrates [13] and high-polymeric yeast RNA [10].

Cell culture. A549 human pulmonary carcinoma cells (American Collection of Cell Cultures, Manassas, United States) were cultured in DMEM (Dulbecco's Modified Eagle Medium, Sigma, Germany) with 10% fetal calf serum (HyClone, United States), 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin in an atmosphere of 5% CO_2 at a temperature of 37°C. Cells from the culture vessels were harvested according to the previously described procedure [14], and 30 μL of the cell suspension (10^6/mL) were plated in 96-well plates per well (ibidi, Ger-